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白纹伊蚊唾液腺重组aegyptin相似蛋白alALP的制备及抗原性分析

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Preparation and Antigenicity Analysis of Recombinant Aegyptin-like Protein of *Aedes albopictus*

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摘要 目的 克隆、表达白纹伊蚊唾液腺重组aegyptin相似蛋白alALP编码基因并分析其抗原性。 方法 运用生物信息学方法分析alALP与aegyptin氨基酸序列（GenBank登录号为ABF18122.1）的同源性、二级结构和抗原肽段。提取白纹伊蚊唾液腺总RNA，根据alALP的基因编码序列（GenBank登录号为AY826121）设计引物，RT-PCR扩增目的基因，亚克隆至pGEX-6P-1质粒，转化大肠埃希菌（E. coli）BL21，经异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）和蛋白质印迹（Western blotting）分析重组蛋白表达情况。重组aegyptin相似蛋白（GST-alALP）经还原型谷胱甘肽（Glutathione Sepharose 4B）亲和层析法纯化后，皮下注射免疫BALB/c小鼠，每次免疫剂量60 μg，共免疫3次，每次间隔2周，制备小鼠抗GST-alALP血清。分别以小鼠抗GST-alALP血清和白纹伊蚊叮咬后小鼠血清为一抗，Western blotting分析GST-alALP蛋白的抗原性。结果 生物信息学预测结果显示，alALP与aegyptin的氨基酸序列具有65.58%的同源性，二级结构高度相似并具有一段保守的抗原多肽。RT?鄄PCR结果显示，alALP成熟肽基因扩增产物约为762 bp。经双酶切、PCR和测序结果显示，重组质粒pGEX-6p-1-alALP构建成功。SDS-PAGE和Western blotting结果显示，经IPTG诱导获得相对分子质量（Mr）约56 000的可溶性重组融合蛋白。Western blotting结果显示，GST-alALP蛋白能被该蛋白免疫的小鼠血清和白纹伊蚊叮咬后的小鼠血清识别。 结论 克隆的alALP成熟肽基因能在原核表达系统中高效表达，且具有抗原性。

关键词： 白纹伊蚊 aegyptin相似蛋白 原核表达 抗原性

Abstract： Objective To clone and express the aegyptin-like protein （alALP） encoding gene from *Aedes albopictus* salivary gland, and analyze its antigenicity. Methods The homology, secondary structure and antigen peptides of alALP and aegyptin protein（GenBank No. ABF18122.1） was analyzed by bioinformatics software tools. Total RNA was extracted from *Ae. albopictus* salivary gland. The coding region of alALP （GenBank No. AY826121） was amplified by PCR. RT-PCR product was digested with restriction enzyme and ligated into a pGEX-6P-1 vector. The recombinant pGEX-6P-1-alALP plasmid was transformed into *E. coli* BL21 and induced by IPTG. The recombinant soluble GST-alALP fusion protein was purified with Glutathione Sepharose 4B. The expression product was analyzed by SDS-PAGE and Western blotting. Mice were immunized each with 60 μg purified GST-alALP at every 2 weeks for 3 times, and mouse anti-GST-alALP serum was prepared. Western blotting assay with mice anti-GST-alALP serum and serum of mice exposed to *Ae. albopictus* bites was used to analyze its antigenicity. Results Bioinformatics prediction results showed that alALP and aegyptin had 65.58% homology with a similar secondary structure, and a conservative polypeptide. The product of RT-PCR was 762 bp. The recombinant plasmid pGEX-6P-1-alALP was confirmed by double restriction enzyme digestion, PCR and sequencing. SDS-PAGE and Western blotting analysis showed that the bacteria containing recombinant plasmid pGEX-6p-1-alALP expressed a soluble recombinant fusion protein（Mr 56 000） after being induced with IPTG. Western blotting analysis revealed that GST-alALP protein was recognized by mouse anti-GST-alALP serum and serum of mice exposed to Ae. albopictus bites. Conclusion Mature peptide gene of alALP can be expressed in prokaryotic expression system, and the recombinant protein shows antigenicity.

Keywords： *Aedes albopictus* Aegyptin-like protein Prokaryotic expression Antigenicity

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